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Wageningen Livestock Research

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Op legpluimveebedrijven worden drinkwateradditieven gebruikt om de natuurlijke weerstand van de hen te bevorderen. Deze middelen lijken als neveneffect een effect te hebben op de levenscyclus van de vogelmijt *Dermanyssus gallinae*; enerzijds doordat de mijten niet naar de hen toe gaan door een afstotend effect van het middel en anderzijds doordat de mijt het bloed van de hen niet kan verteren. Het doel van deze observationele studie was om het mogelijke effect tegen vogelmijt van twee drinkwateradditieven (Hensupp+ en Alphamites DW) te testen. Of deze middelen een registratie als diergeneesmiddel of biocide nodig hebben is geen onderwerp van deze studie geweest. Er is in deze studie alleen gekeken naar het effect van de producten op 1) het produceren van eieren door de volwassen vrouwelijke vogelmijt *D. gallinae* en 2) het vervellen van de nimfen van de vogelmijt *D. gallinae*. Indirect wordt daarmee het effect bekeken van de producten op het doorbreken van de levenscyclus van de vogelmijt. De vogelmijten werden verzameld op 3 commerciële legpluimveebedrijven waar Alphamites DW werd toegepast en op 4 commerciële legpluimveebedrijven waar Hensupp+ werd toegepast. Er werden ook mijten verzameld op een commercieel legpluimveebedrijf dat als controlebedrijf fungeerde. Op dat bedrijf werd geen drinkwateradditief gebruikt maar wel werd silica ingezet om de vogelmijtplaag te beheersen. Recent gevoede vrouwelijke volwassen vogelmijten en recent gevoede vogelmijtnimfen werden binnen enkele uren na het verzamelen op de bedrijven individueel geplaatst in zogenaamde 96 wells microtiterplaten. In deze platen kon bepaald worden of de volwassen vogelmijten eieren produceerden en of de nimfen vervelden. Er is geen significant verschil gevonden tussen het percentage volwassen mijten dat eieren produceerde dan wel het percentage nimfen dat vervelde en afkomstig was van bedrijven waar één van de twee drinkwateradditieven werd gebruikt in vergelijking met de vogelmijten die afkomstig waren van het controlebedrijf. Er werd een trend waargenomen in een lager percentage vervellende nimfen dat verzameld werd op bedrijven waar Alphamites DW werd verstrekt, in vergelijking met het percentage nimfen dat vervelde en verzameld was op het controlebedrijf. Deze proef is uitgevoerd met vogelmijten afkomstig van zeven legpluimveebedrijven waarvan verwacht wordt dat het adviesprotocol van de fabrikant van het betreffende product gevolgd is, maar niet zeker is. Ook is onbekend in hoeverre andere factoren (bijvoorbeeld gezondheidsstatus van de koppel en water- en voerkwaliteit) de resultaten hebben beïnvloed. In deze proef is slechts een klein gedeelte van de mogelijke effectiviteit van de twee drinkwateradditieven getest. Derhalve kan daardoor niets gezegd worden over de totale effectiviteit van de twee drinkwateradditieven op de vogelmijt, zoals o.a. het vogelmijt-werende effect bij de hen door het product.

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Summary

In laying hen facilities drinking water additives are used to support the hens natural resistance. It seems that these products have a side-effect on the life cycle of the ecto-parasitic mite *Dermanyssus gallinae*; either because mites do not approach the laying hens by the products repellent effects or by making the blood indigestible for the poultry red mite. Whether these products do need a registration as veterinary medicine or as biocide was not the subject of this study. The aim of this study was to test the effect of Hensupp+ and Alphamites DW on *D. gallinae* female adults' ability to lay eggs and on the *D. gallinae* nymphs' ability to moult. Here we measured indirect the effect of the products on the lifecycle of *D. gallinae*.

D. gallinae mites were collected at 3 commercial farms using Alphamites DW and at 4 commercial farms using Hensupp+. Mites were also collected at a commercial control farm where no water additives were used, but only a silica product to control *D. gallinae*. Recently engorged female adults and nymphs were individually placed in 96 well plates enabling to determine mite egg production or moulting. No significant differences were found in the percentage of adult mites producing eggs or the percentage of moulting nymphs of the collected mites from the farms with drinking water additives, compared to the mites from the control farm. A trend however was seen for a lower percentage of moulting nymphs from farms which supply Alphamites DW compared to the percentage of moulting nymphs from the control farm.

This observational study was carried out at seven commercial farms of which it can be expected that they carried out the advised treatment protocol, but of which we are not sure. Neither do we know if there were factors present (such as health status of the flock and water and feed quality) which could have influenced the test results. In this test, we determined a small part of the possible effects of the two drinking water additives on red mites and therefore we are not able to draw conclusions about the total effect of the two water additives on the poultry red mite.

1 Introduction

1.1 Background

In Dutch egg laying hen facilities, drinking water additives are applied which have a positive effect on the hens natural resistance. A possible side-effect of two of those products, being Hensupp+ and Alphamite DW, seems to be a reduction in number of poultry red mites. It seems that these water additives influences or changes the blood of the hen making it indigestible for the poultry red mite *D. gallinae* (pers. communication G. Le Bihan). Another effect of the water additives Hensupp+ or Alphamites DW is suggested to be a repellent for the mite. The hens drinking the additive may be less attractive for *D. gallinae* by the product its repellent effect (pers. communication G. Le Bihan). The poultry red mite *Dermanyssus gallinae* is a blood-sucking mite with an almost worldwide distribution and a negative impact on laying hen health, laying hen welfare and egg production parameters (Chauve, 1998). Adult female mites need blood to produce eggs and both the protonymph and the deutonymph need blood to moult to the next stage of the mites lifecycle, becoming deutonymph and adult respectively (Maurer and Baumgartner, 1992). When the nymph moults, an empty "skin" can be found. The yearly costs of *D. gallinae*, production losses and treatment costs, were estimated to be 11 million euro for the Dutch egg producers in 2005 (Van Emous et al., 2005). *D. gallinae* is hard to control as it hides in cracks and crevices in the vicinity of the hens nightly resting place. Every few days the mite emerges from that hiding place to the laying hen to suck blood, mostly when it is dark (Maurer et al., 1988). This specific behaviour, hiding in cracks and crevices and only emerging from there every few days, makes it hard to target the mite with the available conventional contact acaricides and sprayed veterinary medicines, potentially not encountering treated surfaces for several days after application (Maurer et al., 1988). Increasingly stringent pesticide legislation in many parts of the world, as well as the tendency of *D. gallinae* to rapidly develop resistance, further exacerbate this issue (Sparagano et al., 2014). The above mentioned water additives may help in the control of *D. gallinae* in egg laying hen facilities.

1.2 Objectives

The aim of the observational study described in this report is to identify the possible side-effect of two water additives (Alphamites DW and Hensupp+) on the *Dermanyssus gallinae* female adults ability to lay eggs and on the *D. gallinae* nymph's ability to moult, and thus on the effect of the products on the *D. gallinae* life-cycle.

Whether these products need a registration as veterinary medicine or as a biocide is not the subject of this study. This study only focusses on two of the suggested side-effects on *D. gallinae*.

2 Materials and Methods

2.1 Study sites and test products

A laboratory study was performed in November 2016 and August 2017 with *Dermanyssus gallinae* mites collected at eight commercial egg producing poultry farms in The Netherlands (Table 2.1 and 2.3). Three farms were using Alphamites DW as a water additive and four farms were using Hensupp+ (Table 2.2). One farm, using no water additives was repeatedly used as a reference farm, in 2016 and 2017. This farm used silica to control *D. gallinae*. In the test farms one of the water additive products was supplied via the drinking water and it was said that it was applied by the farmers as advised by the producers (Table 2.1).

Table 2.1 Test products; supplier; test period

Test product	Supplied by	Test period	Number of farms
Alphamites DW	Innoresult	November 2016	3
Reference A	-	November 2016	1
Hensupp+	Dosers	August 2017	4
Reference A	-	August 2017	1

Table 2.2 Test products; composition.

Composition	Test product	
	Hensupp	Alphamites
Aromatic plants	x	
Sodium chloride	x	x
Magnesium chloride	x	x
Alcohol	15 %	
Vitamin C		12 g/L
Plant extract		25 ml/L
<i>Origanum vulgare</i>		x
<i>Echinacea purpurea</i>		x
Ginseng		x
Rosa		x
<i>Tanacetum vulgare</i>	x	
<i>Thymus vulgaris</i>	x	

Table 2.3 Test product; farms; location; age hens at start of the test; mites

Test product	Farmer	Location	Age hens (weeks)	Last time used (weeks prior to the test)	Mites
Alphamites	B1	Drenthe	40	nd	Clusters > 1 cm ² ; limited number of mites in traps
Alphamites	R	Flevoland	78	nd	Clusters < 1 cm ² ; limited number of mites in traps; also silica + acid used.
Alphamites	H	Gelderland	31	nd	Mites were lively; many mites in traps
Reference	A: Control 1	Flevoland	nd	-	Mites were lively; mites in traps
Hensupp	D	Noord-Brabant	31	0.4	Clusters > 1 cm ² ;
Hensupp	S	Limburg	47	4	Clusters > 1 cm ² ; limited number of mites in traps
Hensupp	B2	Limburg	90	1	Clusters > 1 cm ² ;
Hensupp	H	Limburg	28	1.5	Clusters > 1 cm ² ; many mites in traps
Reference	A: Control 2	Flevoland	nd	-	Mites were lively; mites in traps

nd= no data available

2.2 Mite sampling

Two different traps were used to collect *D. gallinae* at each farm: the AVIVET trap (Lammers et al., 2017) consisting of one black Tylen tube (length 50 mm, inner diameter of 12 mm, with corrugated cardboard measuring 50 mm x 60 mm x 1mm (figure 2.1)) and the "Rick-stick", a 10 cm long PVC tube with a wooden round bar and a screw in the middle of the bar to fix it in the tube (Emous and Ten Napel, 2007; figure 2.2). The traps (6 AVIVET traps and 2 Rick-sticks per farm) were placed under the perches, as high as possible and more than 3 meter apart from each other. The traps were removed after 3 to 4 days. Each trap was put into a plastic bag, sealed, placed in a cool box and brought to the laboratory for analysis within 2 hours.

Moreover, to ensure the available number of mites, mites were collected from the housing system with a small brush and placed into a vial with a screw cap (35 mm wide and 70 mm long) and put in the same cool box for transport to the laboratory.



Figure 2.1 AviVet trap (Lammers et al., 2017)

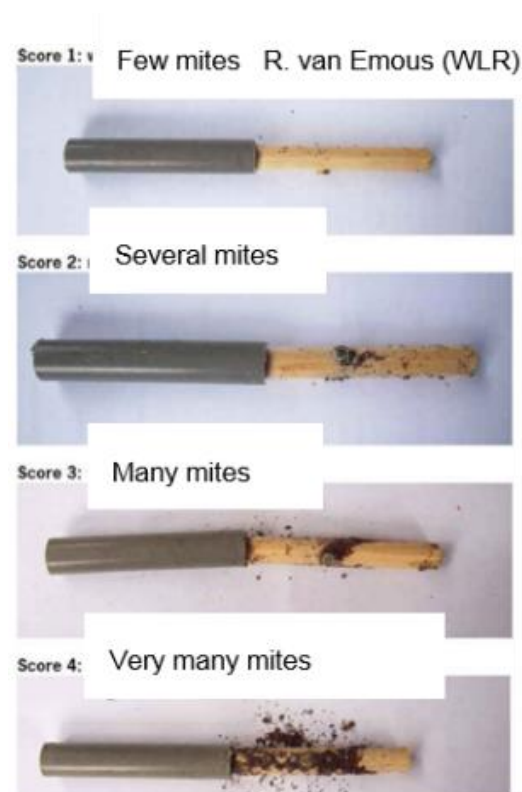


Figure 2.2 Monitoring method Rick-stick (Van Emous and Ten Napel, 2007)

2.3 Mite testing

In the laboratory, per farm 48 female adults and 48 nymphs (protonymphs and deutonymphs) were placed individually in a well of a 96 well flat bottom culture plate (Corning Costar 3596, Sigma-Aldrich). The wells were capped with a EVA cap mat (Micronic MP33000). Only the recently fed (red colour) and lively moving mites were placed in the well. The plates were kept at 25 °C in the dark during four days. The humidity was provided by a vial (35 mm wide and 70 mm long) with water placed in the incubator. For four consecutive days, the wells were scored for eggs (female adults) and moults (nymphs). Also death of the adults and nymphs was scored.

2.4 Statistical analysis

For day four, per farm the percentage of wells with eggs (female adults) was calculated by using the formula: $(\text{the number of wells with eggs per farm}) / (\text{the number of wells with eggs with living adults} + \text{the number of wells without eggs with living adults} + \text{the number of wells with dead adults who did not produce eggs})$.

For day four, per farm the percentage of wells with moults of the nymphs was calculated by using the formula: $(\text{the number of wells with moults per farm}) / (\text{the number of wells with moults} + \text{the number of wells without moults of living nymphs} + \text{the number of wells with dead nymphs who did not moult})$. To determine the differences in either egg production or moulting between the mites from the farms which applied either one of the two water additive products or from the control farm, a logistic regression with over-dispersion was carried out using the logistic regression procedure using the statistical software package GenStat for Windows (17th edition, 2015) (Anonymous, 2006).

3 Results

3.1 Female adults producing eggs

The percentage of wells with eggs produced by the female adults per incubation time and per treatment is shown in table 3.1.

Table 3.1 Percentage egg production of start amount adults.

Incubation time (Days)	Control (%)	Hensupp (%)	Alphamites (%)
1	78.1	76.6	81.3
2	93.2	85.5	95.3
3	95.8	86.1	96.9
4	95.8	88.0	97.9

Figure 3.1 depicts the data from table 3.1.

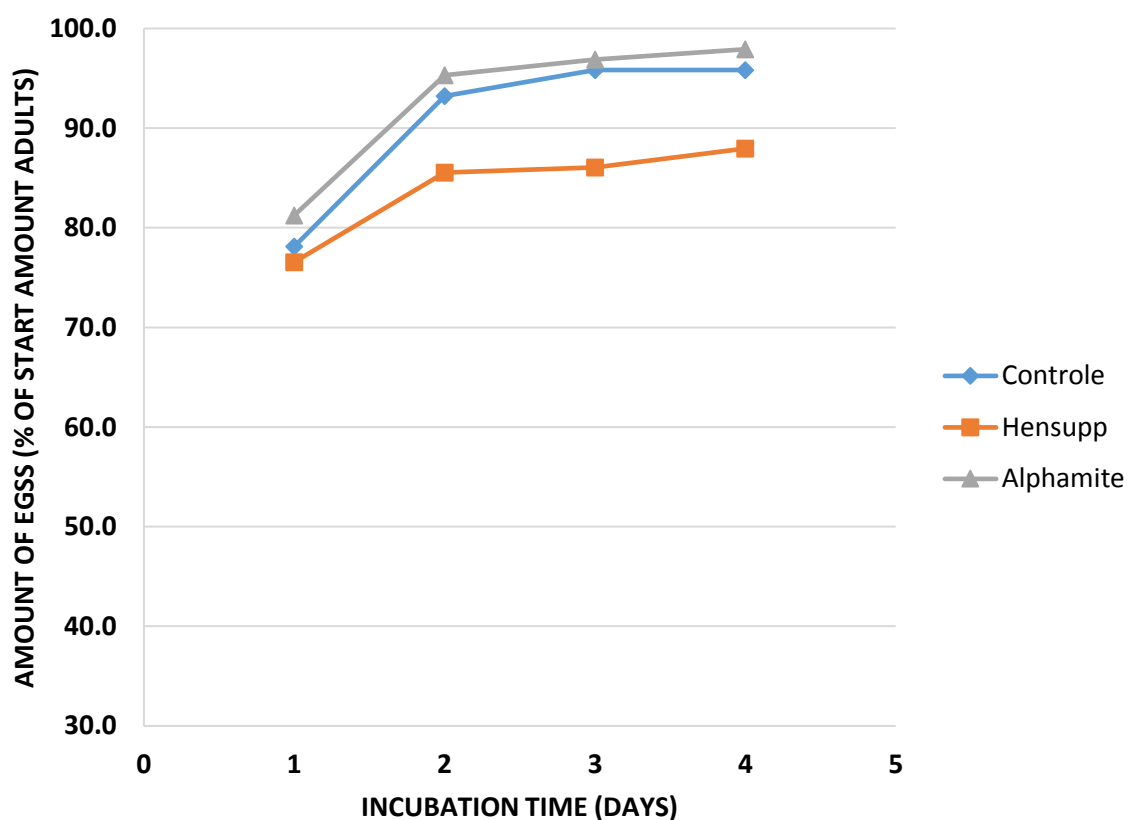


Figure 3.1 Egg production of the female adults (as percentage of start amount of adults) during 4 days of incubation at 25 °C. The results are averaged per quadruplicate.

In this observational study we were not able to show a significant difference ($p > 0.16$) between the egg production of the female adults after four incubation days from the farms applying one of the two water additives compared to the egg production of the female adults after four incubation days from the control farm. Differences could exist by coincidence.

3.2 Nymphs moulting

The percentage of wells with moults produced by the nymphs per incubation time and per treatment is shown in table 3.2.

Table 3.2 Percentage moults of start amount nymphs.

Incubation time (Days)	Control (%)	Hensupp (%)	Alphamites (%)
1	4.2	16.8	4.3
2	74.3	65.3	46.5
3	85.8	82.1	68.6
4	86.4	82.6	72.9

Figure 3.2 depicts the data from table 3.2.

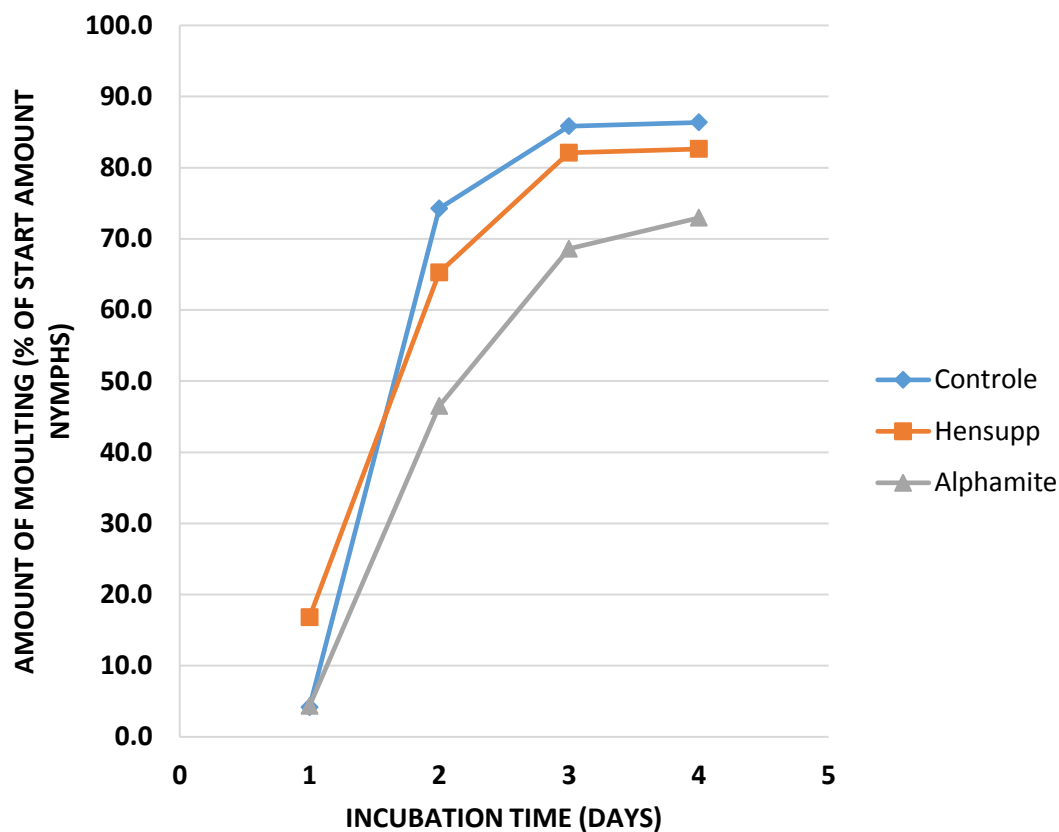


Figure 3.2 Moulting of the nymphs (as percentage of start amount of nymphs) during the four days of incubation at 25 °C. The results are averaged per quadruplicate.

In this observational study we were not able to show a significant difference between the moulting of the nymphs after four incubation days from the farms applying one of the two water additives compared to the moulting of the nymphs after four incubation days from the control farm. However, we found a trend ($p=0.096$) towards a lower percentage of nymphs moulting from farms applying Alphamites DW when compared to the nymphs from the control farm.

4 Discussion

Two water additives (Alphamites DW and Hensupp+) are applied in layer flocks to increase the hens natural resistance. As a side-effect both products seem to reduce the number of poultry red mites. The aim of this observational study is to identify the possible effect of the two indicated water additives on the *Dermanyssus gallinae* female adults ability to lay eggs and on the *Dermanyssus gallinae* nymphs ability to moult. No significant differences were found in the percentage of adults producing eggs or the percentage of moulting nymphs of the mites collected at the farms which applied water additives compared to the mites collected at the control farm. A trend however was seen for a lower percentage of moulting nymphs collected at farms applying Alphamites DW compared to the percentage of moulting nymphs collected at the control farm. A higher number of farms involved in the observational study may have shown if this trend is a coincidence or the real effect of the product.

A higher number of farms will also reduce the influence of one farm on the results of the observational study; as an example the product used at farm B2 (Hensupp+) seem to have a different effect on the mites egg production when compared to the other farms supplying the same product (see Appendix 3). Moreover, with multiple factors affecting the effect of the water additives (e.g. correct use of the advised protocol, sanitary status of the flock, age of the hens, immune status of the hen, other treatments of the hen, water quality, feed quality) a high number of farms is necessary to determine the general effect of the products.

This observational study was carried out with mites from seven commercial farms providing the water additives. It is unknown whether the advised protocol was carried out correctly on these farms. Moreover the health status of flocks and farms and the other factors influencing the efficacy of the products are unknown.

The effect of the water additives on mites are said to be multiple. Amongst others, mites can't digest the blood of treated hens, mites avoid the hen (pers. communication G. Le Bihan), mites become passive or inactive, and eventually the mite dry (pers. communication N. van Lin). In this observational study we only focused on two possible effects of the water additives on the mite's ability to produce eggs (adults) or moult (nymphs) after a blood meal. It was expected that the blood from a treated hen is indigestible for *D. gallinae* and therefor that *D. gallinae* could not produce eggs or moult after a blood meal from the laying hen drinking the water additive. The total effect of the water additives on *D. gallinae* however should be tested in another study with another set-up.

Though we tested the possible side-effects of two water additives on the effect of it on the lifecycle development of *D. gallinae*, the products are sold to increase the natural resistance of laying hens. Whether these products need a registration as veterinary medicine or as a biocide was not the subject of this study.

5 Conclusions

Based on the results of this observational study it is concluded that:

- No significant difference was found in the percentage of female *D. gallinae* adults producing eggs of the mites collected at farms which applied water additives compared to the mites collected at the control farm.
- No significant difference was found in the percentage of *D. gallinae* nymphs moulting of the mites collected at the farms which applied water additives compared to the mites collected at the control farm. A trend however was seen for a lower percentage of moulting nymphs collected at farms applying Alphamites DW compared to the percentage of moulting nymphs collected at the control farm.

The results of this study can be affected by the multiple on-farm factors. With this observational study conclusions about the total side-effect of the water additives on *Dermanyssus gallinae* cannot be drawn as only a small part of the suggested side-effects has been tested. Whether these products need a registration as veterinary medicine or as a biocide was not the subject of this study.

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Appendix 1 Raw data Adults

Testproduct	Farmer	Day	Egg	No_egg	Dead	Nyph	Start amount_adult	%_Egg
Controle	Control 1	1	40	8	0	0	48	83
Controle	Control 1	1	24	24	0	0	48	50
Lphamite	H	1	36	12	0	0	48	75
Lphamite	H	1	41	7	0	0	48	85
Lphamite	R	1	40	8	0	0	48	83
Lphamite	B1	1	39	9	0	0	48	81
Controle	Control 2	1	41	6	1	0	48	85
Controle	Control 2	1	45	2	1	0	48	94
Hensupp	B2	1	22	25	2	0	49	45
Hensupp	D	1	33	13	0	2	46	72
Hensupp	S	1	43	4	1	0	48	90
Hensupp	H	1	48	0	0	0	48	100
Controle	Control 1	2	44	4	0	0	48	92
Controle	Control 1	2	45	3	0	0	48	94
Lphamite	H	2	43	4	1	0	48	90
Lphamite	H	2	48	0	0	0	48	100
Lphamite	R	2	45	2	1	0	48	94
Lphamite	B1	2	47	1	0	0	48	98
Controle	Control 2	2	44	3	1	0	48	92
Controle	Control 2	2	46	1	1	0	48	96
Hensupp	B2	2	28	19	2	0	49	57
Hensupp	D	2	41	4	1	2	46	89
Hensupp	S	2	46	1	1	0	48	96
Hensupp	H	2	48	0	0	0	48	100
Controle	Control 1	3	45	3	0	0	48	94
Controle	Control 1	3	47	1	0	0	48	98
Lphamite	H	3	44	3	1	0	48	92
Lphamite	H	3	48	0	0	0	48	100
Lphamite	R	3	46	1	1	0	48	96
Lphamite	B1	3	48	0	0	0	48	100
Controle	Control 2	3	46	1	1	0	48	96
Controle	Control 2	3	46	1	1	0	48	96
Hensupp	B2	3	28	14	7	0	49	57
Hensupp	D	3	42	2	2	2	46	91
Hensupp	S	3	46	0	2	0	48	96
Hensupp	H	3	48	0	0	0	48	100
Controle	Control 1	4	45	3	0	0	48	94
Controle	Control 1	4	47	1	0	0	48	98
Lphamite	H	4	46	1	1	0	48	96
Lphamite	H	4	48	0	0	0	48	100
Lphamite	R	4	46	0	2	0	48	96
Lphamite	B1	4	48	0	0	0	48	100
Controle	Control 2	4	46	1	1	0	48	96
Controle	Control 2	4	46	1	1	0	48	96
Hensupp	B2	4	30	10	8	0	48	63
Hensupp	D	4	43	1	2	2	46	93
Hensupp	S	4	46	0	2	0	48	96
Hensupp	H	4	48	0	0	0	48	100

Appendix 2 Raw data Nymphs

Testproduct	Farmer	Day	Moult	No_moult	Dead	Empty	Adult	Start amount_nymph	%_Moult
Lphamite	B1	1	2	41	1	0	4	44	5
Lphamite	R	1	4	36	7	0	1	47	9
Lphamite	H	1	1	41	3	0	3	45	2
Lphamite	H	1	1	46	1	0	0	48	2
Controle	Control 1	1	2	43	2	1	0	47	4
Controle	Control 2	1	2	46	0	0	0	48	4
Controle	Control 1	1	1	45	2	0	0	48	2
Controle	Control 2	1	3	44	1	0	0	48	6
Hensupp	B2	1	11	35	1	0	1	47	23
Hensupp	S	1	2	42	3	0	1	47	4
Hensupp	D	1	7	34	7	0	0	48	15
Hensupp	H	1	12	36	0	0	0	48	25
Lphamite	B1	2	30	12	2	0	4	44	68
Lphamite	R	2	23	14	10	0	1	47	49
Lphamite	H	2	1	41	3	0	3	45	2
Lphamite	H	2	32	15	1	0	0	48	67
Controle	Control 1	2	29	16	2	1	0	47	62
Controle	Control 2	2	38	9	1	0	0	48	79
Controle	Control 1	2	37	9	2	0	0	48	77
Controle	Control 2	2	38	8	2	0	0	48	79
Hensupp	B2	2	33	13	1	0	1	47	70
Hensupp	S	2	28	16	3	0	1	47	60
Hensupp	D	2	29	12	7	0	0	48	60
Hensupp	H	2	34	14	0	0	0	48	71
Lphamite	B1	3	36	7	2	0	3	45	80
Lphamite	R	3	29	8	10	0	1	47	62
Lphamite	H	3	25	17	3	0	3	45	56
Lphamite	H	3	37	10	1	0	0	48	77
Controle	Control 1	3	38	7	2	1	0	47	81
Controle	Control 2	3	42	5	1	0	0	48	88
Controle	Control 1	3	43	2	3	0	0	48	90
Controle	Control 2	3	41	2	5	0	0	48	85
Hensupp	B2	3	43	2	2	0	1	47	91
Hensupp	S	3	33	7	7	0	1	47	70
Hensupp	D	3	34	7	7	0	0	48	71
Hensupp	H	3	46	2	0	0	0	48	96
Lphamite	B1	4	37	6	2	0	3	45	82
Lphamite	R	4	31	6	10	0	1	47	66
Lphamite	H	4	29	13	3	0	3	45	64
Lphamite	H	4	38	9	1	0	0	48	79
Controle	Control 1	4	38	7	2	1	0	47	81
Controle	Control 2	4	42	5	1	0	0	48	88
Controle	Control 1	4	44	1	3	0	0	48	92
Controle	Control 2	4	41	2	5	0	0	48	85
Hensupp	B2	4	43	2	2	0	1	47	91
Hensupp	S	4	34	4	9	0	1	47	72
Hensupp	D	4	34	7	7	0	0	48	71
Hensupp	H	4	46	2	0	0	0	48	96

Appendix 3 Results per farm

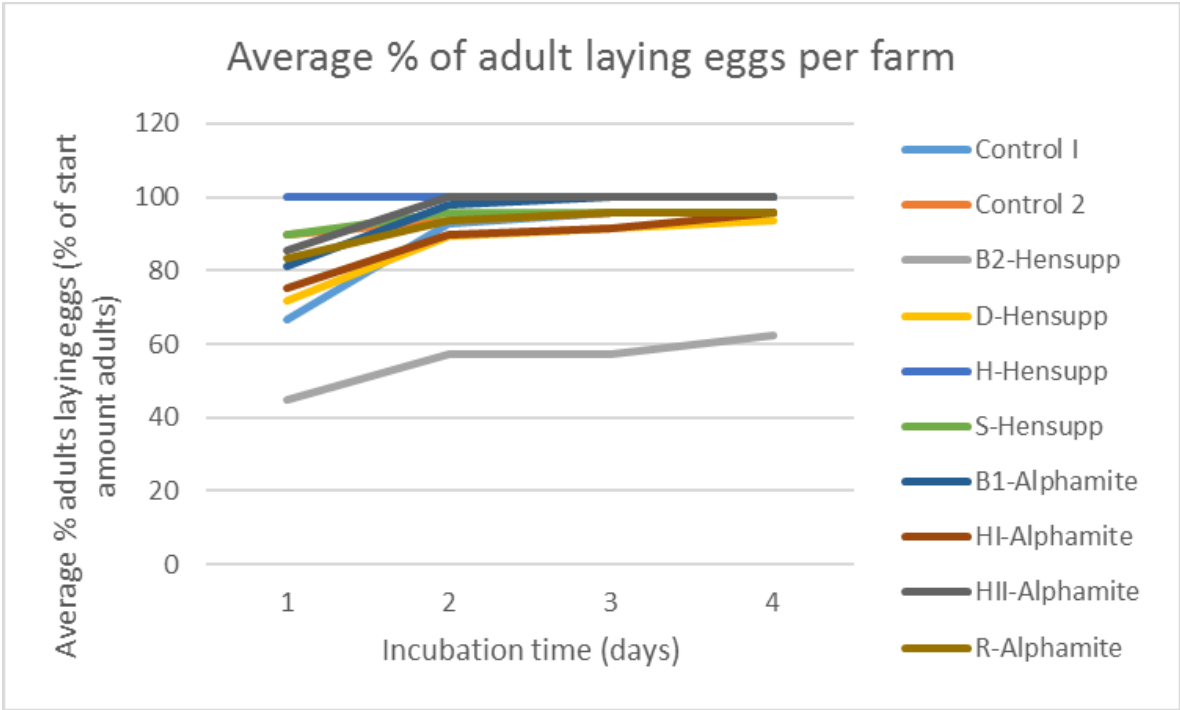


Figure Appendix 3.1 Average egg production of the female adults (as percentage of start amount of adults) per farm during 4 days of incubation at 25 °C.

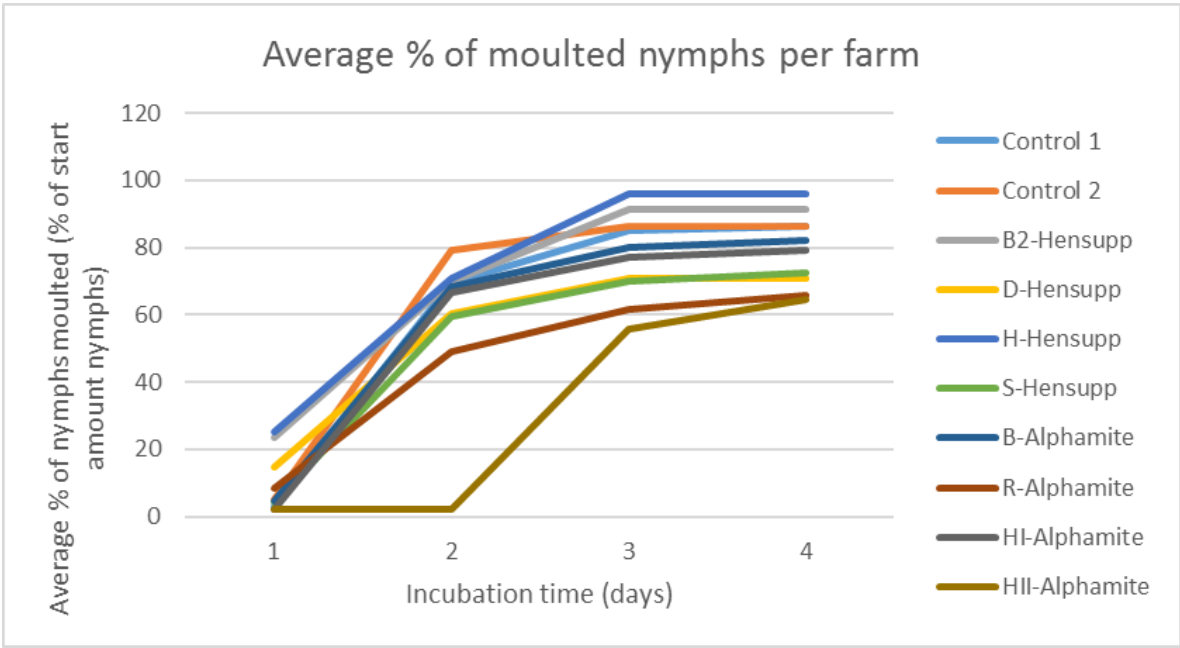


Figure Appendix 3.2 Average % of moulted nymphs (as percentage of start amount of nymphs) per farm during the four days of incubation at 25 °C.

To explore
the potential
of nature to
improve the
quality of life



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